REMARKS/ARGUMENTS

Claims 1-14 are pending. Claim 10 is canceled, and Claims 1, 2, 9, 11 and 13 are amended. No new matter is added. Support for the amending language of Claim 13 may be found in the specification at page 7, line 6. Applicants respectfully request reconsideration of the rejections, and allowance of Claims 1-9 and 11-14.

Claim 9 was objected to for have an abbreviation that was not defined. Claim 9 has been amended to read dimethyl sulfoxide. Applicants request withdrawal of the objection.

Claim 2 has been rejected under 35 U.S.C. 112, first paragraph. Without conceding to the correctness of the rejection, and without prejudice to renewal or refilling of claims having the original scope, claim 2 has been amended wherein substrates for PDI activity are insulin and HIV gp 120. Withdrawal of the rejection is requested.

Claim 1 was rejected to as being unsubstantiated by the consensus of the art for prolyl 4-hydroxylase and hypoxia-inducible factor. This point is mute in light of the amendment to claim 2 and applicant requests withdrawal of the rejection.

Claims 1-14 have been rejected under 35 U.S.C. 112 second paragraph. Claim 1 has been amended to indicate one optical density measurement is taken after the reaction mixture is stopped with hydrogen peroxide. The optical density reading is compared against a negative control, which is now reflected in claim 1 as amended. Claims 1, 9 and 13 have been amended to recite "said reaction mixture". Withdrawal of the rejection is requested.

Claim 13 has been amended to recite the phase "recombinantly produced" which is supported on page 7 line 6 of the present specification. This amendment clarifies what the Applicant mean by the previous term 'defined source of PDI activity'. Withdrawal of the rejection is requested.

Claims 1-14 were rejected under 35 U.S.C. 103 as unpatentable over Bonfils *et al.*, (Eur. J. Biochem., 1998, 254:420-427) in view of Qvist *et al.* (U.S. Patent no. 6,110,689), Cahoon *et al.* (WO 00/22100) and Moussebois *et al.* (U.S. Patent no. 4,397,960), Ryser (WO94/04185); and Dunlay et al. (U.S. Patent no. 5,989,835). Applicants respectfully submit that the presently claimed invention is not made obvious by the cited combinations of references.

The presently pending claims are all dependent on Claim 1, which has been amended to recite

the limitations of originally filed Claim 10, wherein a plurality of assays are performed in parallel. Claim 11 further recites that the assays are performed in a microtiter plate. The rejections previously applied that excluded Claim 10 are therefore made moot and are not further considered.

The present invention provides a useful, high-throughput turbidometric assay for determining PDI activity. The claimed assay requires a specific stop reagent, and is performed in parallel reactions. In some claimed embodiments, the reactions are performed in a microtiter well. The cited art does not make obvious this method. Applicants have further provided herewith a Declaration under 37 C.F.R. 1.132 in support of the arguments provided herein.

As described by Dr. Huang, the key to the success of the claimed assay is an accurate mixing step, which has to be brief and thorough, and a clear stop to the reaction. If the mixing is not brief, one will miss the initial reaction phase. If the mixing is not thorough, the reaction kinetics can show a lag because the reactants did not "meet" each other instantly after mixing, thereby confounding the resulting values obtained. And without a distinct stop, which does not alter the readout of the assay, it is difficult to perform a high throughput assay, with multiple parallel reactions. The prior art does not teach one of skill in the art how to reasonable perform such an assay.

Bonfil discloses a method for measuring PDI activity. The method is performed in a cuvette via the measurement of disulfide reduction of insulin. No stop reagent was added.

The modification of the assay from a single cuvette to a parallel, high throughput assay system would not have been obvious to one of skill in the art. The turbidometric assay described in the reference is extremely difficult to perform accurately, even in a cuvette, one reaction at a time, performed by a well-skilled experimenter. It is particularly difficult to obtain accurate results using these techniques in a parallel assay format. As described in the attached Declaration, it is not straightforward, nor is it obvious to adapt a conventional cuvette assay to a parallel well plate assay due to the nature of a kinetics assay where brief and thorough mixing is essential, the restrictions placed on high throughput screening campaigns; and the requirement for an accurate stop reagent. Prior to the teachings of the present invention and the modifications required to generate the functional assay under HTS conditions, it was unclear whether a high through put assay could be achieved.

It is also not obvious which inhibitor or stop reagent to use for the PDI assay. Hydrogen peroxide could disrupt the aggregated insulin chain due to oxidation of the SH groups back to the oxidized form and thus change the OD reading. In addition, hydrogen peroxide is an aggressive oxidant and could react with the enzyme and substrates. Whether this stop reagent would work or not required specific experimental demonstration and inventive input, and could not have been expected based on the teachings of the prior art.

The citation of Qvist *et al.* is provided for teaching an assay wherein the enzyme reaction is run in an automated microtiter plate reader. Applicants respectfully submit that the secondary reference does not overcome the limitations of the primary reference. While Qvist *et al.* teach a high throughput assay, it is an equilibrium binding assay, which does not require careful synchronization of multiple wells, does not require a stop reagent to provide for accurate test results, and does not utilize PDI activity. The combination of references fails to teach the claimed invention.

Cahoon *et al.* provides the sequences of a number of arthropod protein disulfide isomerases, and discloses the well-known fact that PDI catalyzes the rearrangement of intrachain and interchain disulfide bonds in proteins to form native structures, and that PDI needs reducing agents or partly reduced agents. Applicants respectfully submit that the secondary reference does not overcome the limitations of the primary reference. Cahoon *et al.* fail to teach the adaptation of the specific PDI turbidometric assay to a parallel format, and further fail to teach the specific stop reagent utilized by Applicants in the subject assay.

Ryser *et al.* pertains to a method of altering reduction of disulfide bonds of membrane-bound macromolecules, particularly proteins, by inhibiting the reductive function of cell membranes, particularly the plasma membrane, which is capable of cleaving disulfide bonds in membrane-bound proteins. The reference demonstrated that the reductive function of cell surface membranes is catalyzed by protein disulfide isomerase (PDI). Applicants respectfully submit that the secondary reference does not overcome the limitations of the primary reference. Ryser *et al.* fail to teach the adaptation of the specific PDI turbidometric assay to a parallel format, and further fail to teach the specific stop reagent developed by Applicants. The reference teaches the PDI activity in living cell membranes, not high throughput assays.

Dunlay *et al.* provides background information on drug discovery and high throughput screening of compound libraries. The cited patent relates to a computer controlled optical-mechanical system for rapidly determining the distribution, environment, or activity of fluorescently labeled reporter molecules in cells for the purpose of screening large numbers of compounds for those that specifically affect particular biological functions. Dunlay *et al.* fail to teach the adaptation of the specific PDI turbidometric assay to a parallel format, and further fail to teach the specific stop reagent developed by Applicants.

Moussebois *et al.* is concerned with immunoassays, more particularly with immunoassays involving the binding properties of immunoglobulins, and with certain reagents useful in such assays. It may be noted that such assays differ significantly from those of the present invention, as they are

equilibrium binding assays not kinetic assays. Moussebois *et al.* state that "any DTT can be inactivated by oxidation with hydrogen peroxide."

Applicants respectfully submit that it is not obvious to one of skill in the art that hydrogen peroxide would be useful as a stop reagent in a highly parallel turbidometric assay. The fact that the reagent inactivates DTT is only the first step. For use on the specific assay claimed by Applicants, because it is a kinetic assay (not an equilibrium assay) stopping the reaction is critical to the invention. The reaction must be stopped in a short period of time, using a reagent that does not interfere with the optical density, which stops the chemical as well as the enzymatic reaction; and which allows the samples to be stably stored for at least about 1 day.

Moussebois *et al.* do not lead one of skill in the art to conclude that H_2O_2 would be useful in the practice of the present invention. As discussed above, there are many considerations apart from oxidation properties of the reagent. Further, one of skill in the art would not have been motivated to select H_2O_2 as a reagent for these reactions because of the oxidizing affects on insulin sulhydryl groups, which could have resulted in changes to the optical density on the reaction.

In summary, the presently claimed invention is not made obvious by the cited combination of references. The change from a cuvette assay to a parallel assay required considerable development, including the selection of an appropriate stop reagent. During the course of development of the claimed methods, there was many attempts to stop this assay with numerous different agents including; acids such as hydrochloric acid, acetic acid, sulfuric acid and phosphoric acid, alkali such as sodium hydroxide, potassium hydroxide, and PDI inhibitor bacitracin. These experiments were unsuccessful in stopping the reaction in a short period of time, using a reagent that does not interfere with the optical density, which stops the chemical as well as the enzymatic reaction.

In view of the above amendments and remarks, Applicants respectfully submit that the present invention meets the requirements of 35 U.S.C. 103. Withdrawal of the rejections is requested.

Conclusion

In view of the above amendments and remarks, this application is considered to be in good and proper form for allowance and the Examiner is respectfully requested to pass this application to issuance.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number AGYT-037.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: May 23, 2005

By: Pamela Sherwood
Pamela J. Sherwood
Registration No. 36,677

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, California 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231